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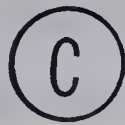
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THE UNIVERSITY OF ALBERTA

The Catecholamine Excretion During Rest,
Maximal and Submaximal Exercise in Active,
Semi-Active and Sedentary Subjects

by



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A THESIS

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ABSTRACT

The urinary catecholamine excretion of active, semi-active and sedentary subjects after rest, maximal and submaximal exercise were compared. A secondary purpose was to establish and use a chromatographic method for the catecholamine analysis of the urine.

Significant differences were found between the excretion of catecholamine during maximal and submaximal exercise ($p < .01$). A decrease in non-active after maximal exercise from the resting values ($p < .01$) and an increase in submaximal exercise catecholamine excretion from the resting values ($p < .01$) were noted. No significant difference was found in the resting excretion.

In the chemical preparation for the chromatographic determination of the catecholamines, alumina was used for the adsorption of the catecholamines at a pH 8 - 8.6. Acetic acid was used as the eluting reagent. The free catecholamines were made volatile by converting them to trimethylsilyl derivatives. The trimethylsilyl derivatives were then injected into the chromatograph. This method gave 64% recovery and results comparable to other studies.

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CHAPTER I

INTRODUCTION

Catecholamines are secreted by the adrenal medulla and the nerve endings of the sympathetic nervous system (SNS*) (34, 44, 65). The SNS originates in the thoracic and lumbar regions of the spinal cord. Nerves from this system go to different organs in the body: for example, heart, lungs, peripheral blood vessels, gonads and eyes. Catecholamines are thus released at these organs, and by the adrenal medulla into the blood.

Because NE is released at the SNS fibres, the system is sometimes called the adrenergic system. At the nerve endings of the postganglionic fibres are found chromaffin cells which are responsible for the storage of NE and for the transmission of the impulse to an organ. These cells are also concentrated in the adrenal medulla (65). The mediator for the release of NE by the chromaffin cells is ACh, which is also released at the pre- and postganglionic fibres of the SNS and PSNS (34, 65).

Several factors control or affect the release of catecholamines; stress (13, 24), body position (61, 70, 77), temperature (42, 45, 62) and exercise (13, 35, 36, 47, 50, 72).

* In this paper the following abbreviations will be used: Sympathetic Nervous System (SNS); Epinephrine (E); Norepinephrine (NE); Acetylcholine (ACh); Parasympathetic Nervous System (PSNS); Maximal Oxygen Uptake (MVO_2).

During exercise the excretion of catecholamines increases due to SNS activation of the smooth muscles in the intestines and a redistribution of blood to the active muscles (31). The severity and duration of the exercise will determine the rate of secretion and release of catecholamines (35, 72).

The adrenal medulla secretes E and NE, neither of which are absolutely necessary for life. Adrenalectomized animals have been shown to live normal lives if they were not exposed to undue stress (9). However, if E and NE are not available to fit the need of the body, stress and exercise can be fatal. During rest a marked increase in the secretion of catecholamines can indicate either that the person is under stress (24) or that a tumor has been formed in the chromaffin cells (20). Urinary catecholamine excretion values up to one hundred times that of the normal is a reliable diagnosis of pheochromocytoma (64).

The effect of exercise upon urinary catecholamine excretion has been studied widely (6, 15, 31, 39) and a comparison of these effects demonstrates a marked similarity between sedentary and trained subjects. The effects of catecholamines on the body are closely related to the adjustments made by the body during exercise. Catecholamines increase the heart rate and cardiac output. NE increases the systolic and diastolic blood pressure, while E increases only the systolic pressure. Catecholamines diminish the renal blood flow (20), causing a decrease in the formation

and filtration rate of the urine output (31). Catecholamines cause a constriction of the skin capillaries, spleen and blood vessels of the lungs and bronchioles.

In the tissue of these organs there are alpha and beta receptors. Catecholamines act on these receptors to cause either constriction or dilation of the blood vessels, depending upon whether the alpha or beta receptors are activated. Activation of alpha receptors by E causes a constriction, whereas activation of the beta receptors by E and NE causes a dilation of the circulatory system. The constriction of these organs will thus be brought about because of an abundance of constriction fibres and will respond vigorously to E and NE (68). Epinephrine causes vasodilation of the muscle capillaries (16) and a consequent increase in blood flow, which is necessary to supply oxygen and nutrients to the muscles, and for the removal of waste products from the muscles.

Urine excretion depends on fluid intake (8), humidity, emotional factors (9), physiological state and temperature (9). Perspiration and the excretion of E and NE (20) during exercise will cause a decrease in the urine output (9). The blood flow to the kidney is about 70% less during exercise than during rest (31). At loads ranging between 150 - 300 kpm. on the bicycle ergometer, which is a submaximal load, and loads to 900 kpm. for a maximal task, the renal clearance will progressively decrease. This will in turn cause a decrease in the urine output. After maximal exercise, no

marked change in the urine catecholamine composition will be apparent because of the decreased filtration rate and a resultant increase in the absorption of the tubules (59). Renal clearance has been shown to not change significantly until the exercise performed was heavy (31).

Upon considering the effects of catecholamines on the body, and comparing them with the effects of exercise, the questions arise: Is there a relationship between the excretion of catecholamines during work at different loads and does a difference exist between sedentary, semi-active and active persons?

Studies have been carried out on the secretion of catecholamines in the blood (30, 67) and excretion in the urine (36, 40, 74) after exercise. Some studies used healthy young subjects (40, 67, 74) but no specific state of fitness was mentioned or no differentiation between loads was made. Other studies used subjects with different levels of fitness (47,73) but in one case no direct measurement of the loads was available (47) and in the other case (73) no statistical comparison was made of the loads and catecholamine excretion. The exercises varied from cross-country races, sprints to pedalling a bicycle ergometer. No study related both maximal and submaximal loads to the excretion of catecholamines and no consistency existed in the exercises or the loads.

The basic purpose of this study was to compare urinary catecholamine excretion after maximal and submaximal exercise in active, semi-active and sedentary subjects. A secondary

purpose was to establish a new method of assaying urinary catecholamines using gas chromatography.

CHAPTER II

SURVEY OF RELATED LITERATURE

Catecholamines are formed in the chromaffin cells of the adrenal medulla and the nerve endings of the SNS, and comprise approximately one-third of the weight of the adrenal medulla. In the SNS the only primary catecholamine is NE (51, 56), while in the adrenal medulla the primary catecholamines are E and NE (51). The catecholamines are secreted by the adrenal medulla into the bloodstream and then transported to different organs. Norepinephrine is also synthesized in the postganglionic fibres of the SNS and released as a neuro-transmitter (84).

The basic substance in the formation of the catecholamines is an amino acid, phenylalanine. The phenylalanine is converted in the liver to tyrosine. This conversion is activated by the enzyme hydroxylase (84). The tyrosine is taken up from the bloodstream into the SNS endings by active transport (84). In the SNS endings the tyrosine migrates to the mitochondria where it is converted to dopa in the presence of the enzyme tyrosine hydroxylase (84). The dopa migrates back to the cytoplasm where it is converted to dopamine. This action is catalyzed by an enzyme L-dopa decarboxylase (51). Dopamine is a minor constituent of the chromaffin cells in the medulla but is about 50% of the catecholamine constituent of the SNS fibres, the other 50% is NE (65).

Mono-amine oxidase inhibitors elevate the concentration of dopamine in certain areas of the body (46) such as the mucous of the jejunum, liver (65), hypothalamus (44) and lungs (37).

Within the granulated vesicle, which contains the enzyme dopamine oxidase, the dopamine is converted to NE which is stored in the chromaffin cells in the SNS fibres (84). Small amounts of the catecholamines are released continuously into the bloodstream under normal conditions. The amounts delivered to the organs are dependant on the blood flow to each organ. Catecholamines in the blood, if not used, undergo certain changes. In organs such as the liver, there are high concentrations of catechol-O-methyltransferase and the catecholamines in the bloodstream are converted to their O-methylated products, metanephrine and normetanephrine by this enzyme (83). These products are then excreted in the urine or converted to Vanilyl-mandelic acid and methoxy hydroxy phenyl glucol and excreted as such (51). The drug cocaine decreases the capacity of the tissue to bind with NE (44) by decreasing the permeability of the membrane of the vesicle granules. Other substances that affect the uptake and storage of catecholamines are: tyramine, which causes an increase in blood catecholamine and a decrease in tissue catecholamine (44); reserpine, which depletes the SNS catecholamine stores (1); A.C.T.H. (76); insulin (19); alcohol (57) and methimazole (45).

The first indication of a hormone in the adrenal gland

was discovered by Vulpian in 1856. Between 1900 and 1910, E was isolated, synthesized and purified (65). In 1906 E was recommended as a remedy for hypertension (69) but not recognized as such before 1949 (65). In 1950 and 1955 it was suggested that if the blood pressure falls below 50 mm. Hg a tumor may have been formed in the chromaffin cells and would be indicated by a urinary catecholamine excretion (20).

Investigations (10, 73, 74, 77) on the effect of administration, either orally or intravenously, of catecholamines have been carried out. After administration, the amount excreted increased significantly from that prior to the administration. This indicated that the body needs a definite amount of catecholamines and the excess is excreted. Eight per cent of the NE was excreted 10 minutes after administration. In rats, 70% of the administered catecholamines were excreted during the first three hours (46).

When it was certain that catecholamines were excreted normally in the urine the interest moved to other aspects, and studies to determine the catecholamine excretion during various body conditions were carried out. Such conditions studied were: body position (50, 68, 71), physical stress (32), temperature (23, 42, 65), mental stress (13, 24) and exercise (13, 35, 36, 47, 50, 72).

An increase was found in catecholamine excretion in healthy persons when they stood up and when tilted from recumbancy to 75 degrees (68, 78). An experiment (71) with ten females, some recovering from operations and others with

mammary cancer, indicated significant increases during tilting; in some cases to twice the resting value. Von Euler showed a significant increase in the catecholamine excretion when changing from a resting to a standing position (68). The reason for the increase was found to be the activation of the SNS (51). It would also be expected that stress of muscular restraint would be associated with both neurogenic-sympathetic and medullary activity with a resultant increase in the excretion. An investigation (45) of the effect of cold adaptation on the excretion of urinary catecholamines showed a significant increase during the adaptation process. In normal, healthy male subjects, exposure to cold revealed a rapid increase in the catecholamine excretion (63). Evidence of a decrease in the urinary excretion in dogs was found with temperature changes from 21 - 48.9°C. (50).

During a Finnish bath a significant increase was found in the urinary catecholamine excretion in human subjects (43). The marked increase in NE was due to an increased SNS activity. During a sauna bath, with temperature between 70 to 80°C., the body temperature of a person tends to remain constant. By doing so, the peripheral blood vessels were dilated, inactivity of the skeletal muscle and a fairly high metabolic rate occurred with a resultant increase in the perspiration excretion. The dilation of the blood vessels was due to SNS activity initiated by the hypothalamus. While SNS activity resulted in the increase of

NE, the increase in E was probably due to adrenal medulla activity (43).

During emotional stress an increase was found in the excretion of the catecholamines (13, 24). An experiment with 23 healthy males and 9 healthy females showed that the excretion of E increased while that of NE was stable. Before making a jump, paratroopers showed an increased excretion of catecholamines in the urine. The jump was performed during the night to provoke mental stress (13). Attempts to link personality traits to the excretion of catecholamines resulted in negative correlations. Measurements were made to determine the catecholamine excretion in schizophrenics treated with large doses of chlorpromazine (15). A comparison was made between treated and untreated fit persons and the investigator found that the excretion was lower in the untreated fit patients.

As early as 1922 (36) attempts were made to determine whether catecholamines were excreted during exercise. Epinephrine and NE were found to be excreted as free or conjugated (35). An increase in the excretion of conjugated catecholamines was observed during a cross-country run. Four urine samples were taken: one 45 minutes before, a second 15 minutes before, the third 15 minutes after the start of the exercise, and the final sample 15 minutes after the end of the race. All samples differed significantly from that of the resting excretion level.

While training for sports events, an increase in the

urinary catecholamines was found in three middle distance runners (47). The exercises consisted of dashes and gymnastics. Urine samples were collected 1.5 - 2 hours before the start of the race. Another sample was taken 2 - 2.5 hours after the exercise. The excretions before and after the exercise showed significant differences. In all cases the excretion was lower after the exercise than during the exercise (47).

During a wood-cutting competition, marked increases were found in the urinary catecholamine excretion for five subjects (47). The amount of catecholamines excreted during ground work performed by fifteen paratroopers showed highly significant differences between resting excretion and the excretion after work (13).

The excretion of catecholamines in the urine of healthy males was studied by Von Euler (73). Urine samples were collected 1 - 2 hours before and as soon as possible after the exercise. The exercises lasted from 5 - 70 minutes; thus, from maximal to submaximal work. The excretion was compared to the oxygen uptake and the work accomplished. The subject with the highest oxygen uptake also showed the greatest increase in catecholamine excretion. During work at a load of 1,950 kpm. the increase was the highest. The percentage of E in the total excretion did not change, indicating an increased activity of the SNS. From the results there appears to be a relationship between workload, oxygen uptake and catecholamine excretion.

Early methods of assaying catecholamines relied on intravenous injection in cats, rats and rabbits of a known amount of catecholamine and then comparing the unknown to the known amounts (51). As interest in catecholamines increased, so did the assaying methods improve. Chemical methods, which were not reproducible, were used in some cases. These methods were based on the oxidation of the catecholamines with iodine, permanganate, arsenomolybdic acid and naphthaquinine (51). Recently fluorometric methods have been employed with great success (10, 15, 70, 73). Fluorometric methods which are used most frequently are the tri-hydroxy indole and the ethylene diamine method.

In the tri-hydroxy indole methods the catecholamines are oxidised (with a weak acid) to adrenochrome and noradrenochrome. These are then converted to adrenolutin and nor-adrenolutin by oxidation and then estimated by a yellow-green fluorescence in ultra violet light. The ethylene diamine method is based on the acetylation of the catecholamines which are then passed through an alumina column to be adsorbed. The alumina is washed with ethelenediamine hydrochloride. The estimation is performed by measuring the fluorescence of the condensation products. When urine was kept for a long time there was a tendency for the formation of a strong non-specific fluorescence in the urine sample (3). Several substances can be measured with the fluorometric method and since urine contains many related substances an overestimation can be obtained.

The introduction of the high-performance gas chromatograph opened the possibility of assaying the catecholamines by this technique. The sensitivity of the instrument and the inherent separation of the substance in the urine extract offered the possibility of a more accurate and reliable method.

The gas chromatograph extraction procedure used for catecholamines was basically the same as that used for fluometry. At pH 4 some of the interfering substances were adsorbed by the alumina while the catecholamines remained in the urine (51). At an alkaline pH, catecholamines can very easily be adsorbed onto alumina (51). Aluminum oxide (2, 58) and aluminum hydroxide (51) have been used in most cases for the adsorption of the catecholamines at a pH of 8 - 8.6 (2).

To recover the catecholamines from the alumina, diluted acetic acid was used. The free catecholamines were converted to a more volatile compound by converting them to trimethylsilyl ethers (14, 61). This conversion was accomplished with hexamethyldisilazane, pyridine being used as the solvent (61). Only small quantities of the solution are required for injection into the gas chromatograph. The reaction between hexamethyldisilazane and pyridine and the free catecholamines occurred slowly, therefore trimethylchlorosilane was used as a catalyst.

A wide variety of columns are used in gas chromatography. The support used was Diataport S. These columns are treated with hexamethyldisilazane to

prevent the polar groups from binding with the support material (54). The coating of the support is usually with a silicone rubber.

CHAPTER III

METHOD AND PROCEDURES

The subjects used in this study were students of the University of Alberta. The active subjects were chosen from the varsity wrestling team. First year students enrolled in a Physical Education program acted as the semi-active group, while the sedentary group was selected from students who did no exercise. All subjects were healthy normal males.

All subjects were tested on a Monarch Bicycle Ergometer to obtain the MVO_2 by means of the Astrand-Rhyming Nomogram (6). This test has been proven to give a good indication of the ability of the body to maintain internal equilibrium (5, 39, 55). The heart rate was recorded on a Sanborn 500 Electrocardiogram. The exercise was performed at a speed of 50 rpm. until the heart rate reached a steady state. If the heart rate was below 125 bpm. or above 180 bpm. the load was increase or decreased on the second test (4). Resting urine samples were obtained prior to this test.

Approximately two weeks after the first test a maximal MVO_2 test was performed. The load at which the heart beat reached a steady state for the first test was taken for the initial load. After 1 minute of exercise at a speed of 50 rpm., the load was increased by one-third of the initial load. At this level the subject exercised for another minute when the load was increased by another one-third of

the initial load. At this load the subject exercised until exhaustion, the work time being noted in seconds. Ten minutes after the exercise, urine samples were obtained.

By means of the Astrand-Rhyming Nomogram (6) the work load for the submaximal exercise was obtained. The load amounted to the equivalent of 70% of the MVO_2 . At this load at a speed of 50 rpm. the subject exercised until exhaustion. The time was noted in minutes. Urine samples were obtained 10 minutes after the exercise. The subjects were asked to void an hour before obtaining the resting sample and an hour prior to the exercise. The urine samples were frozen in dry ice and 95% ethanol.

A sample of the thawed urine was centrifuged for 5 minutes at a speed of 2,000 rpm. Ten ml. of the centrifuged sample were transferred to a 50 ml. glass beaker. The pH of the urine was adjusted to 4 with 1 N hydrochloric acid with continuous stirring and 5 g. alumina were added. The sample and the alumina were centrifuged for 5 minutes at a speed of 2,000 rpm. The urine was transferred to a 50 ml. glass beaker and the pH was adjusted to 8 - 8.5 with 1 N sodium hydroxide and then .5 g alumina was added with continuous stirring. The pH was readjusted and maintained at 8 - 8.5 with 1 N sodium hydroxide and continuously stirred.

The sample and alumina were quantitatively transferred to a centrifuge tube and centrifuged for 5 minutes at a speed of 2,000 rpm. The urine was discarded and the alumina washed

several times with 15 ml. of distilled water. The catecholamines were then eluted with 5 ml. of .25 N acetic acid. The acetic acid and alumina were shaken for 3 minutes with an electric shaker to ensure complete elution. The sample was allowed to sit for 10 minutes and then centrifuged for 5 minutes. The acetic acid elute was transferred to a test tube with a ground joint and dried on a vacuum line.

Two hundred μ l. of a mixture of pyridine, hexamethyldisilazane and trimethylchlorosilane (2:1:1) were added to the dried sample. The reaction mixture was allowed to sit for 1 hour before addition to the dried catecholamines. After the addition of the mixture the sample was shaken for 3 minutes with an electric shaker and allowed to sit for 1 - 2 hours. Two μ l. of the sample were injected into a F & M model 402 High Efficiency Gas Chromatograph. Chromatograms were obtained and the amount of catecholamines present in the 10 ml. sample was calculated against a known standard. The standard was made up by dissolving 10 mg. of dry E in 1 ml. of the reagent mixture.

The chromatogram was temperature programmed at 5°C./minute from 150 - 220°C. An 8-foot column packed with 3.8% UC-W98 on Diataport S was used. A single peak was obtained for both E and NE. Thus, all values quoted represent the sum of E and NE, and since NE urinary excretion is larger, the peak represents mostly NE. The inability to distinguish E and NE was not a problem in the present project and was counterbalanced by the simplicity of the derivative

forming procedure.

In order to test the means of the catecholamine excretion for the three groups, initial means were adjusted and an analysis of covariance was used. The analysis was designed to test the significance of the difference between the adjusted means for the three groups with the three conditions of rest, submaximal and maximal work on the bicycle ergometer (22, 27, 80). Missing values were computed by a method designed by Goulden (29).

CHAPTER IV

RESULTS

Table I shows six recoveries of standard catecholamines after addition of a known amount of E to urine.

TABLE I: RECOVERIES OF CATECHOLAMINE STANDARD AFTER ADDITION TO URINE

Sample (number)	Recovery (%)
1	70
2	74
3	57
4	52
5	63
6	67
$\bar{x}$	64

The mean of the total recoveries was 64%. This was higher than the results reported in a previous study (61). Although only 64% of the catecholamines were recovered, the difference was not used in computing the amount excreted. The retention time for the catecholamine peak occurred after 15 minutes at a temperature of 212°C. The peak heights were measured and multiplied by the calibration factor obtained from the chromatograms of the standard to give the sample contents.

For the seventeen subjects who took part in the experiment, 49 urine samples were obtained. Subjects 1 and 2 of

the active group did not void after submaximal and maximal exercise respectively. The pH of the urine ranged from 4.5 - 7.5. In three samples, which were alkaline, no catecholamines were found.

TABLE II: CATECHOLAMINE EXCRETION FOR ACTIVE, SEMI-ACTIVE AND SEDENTARY GROUPS AT REST*

Subject	Active	Semi-Active	Sedentary
1	0.13	0.43	0.11
2	0.35	0.50	0.29
3	0.17	0.16	0.20
4	0.50	0.21	0.08
5	0.55	0.17	0.40
6	0.31	-	0.02
$\bar{x}$	0.34	0.28	0.18

* All values are given in $\mu\text{g}/10 \text{ ml.}$ urine

Table II indicates that the catecholamine excretion during rest ranged from .02 - .55 $\mu\text{g}/10 \text{ ml.}$ The mean resting excretions are as follows: active .34 $\mu\text{g}/10 \text{ ml.}$, semi-active .28 $\mu\text{g}/10 \text{ ml.}$ and sedentary .18 $\mu\text{g}/10 \text{ ml.}$ The volume of urine excreted for the resting samples ranged from 25 - 109 ml. The means for the three groups were: active 45 ml., semi-active 64 ml. and sedentary 81 ml. Although the subjects were asked to void one hour prior to the collection of the samples, values such as 25 and 109 ml. were still obtained. When the resting catecholamine/10 ml. samples were converted to total catecholamine excretion the following means were found: active 1.54 μg , semi-active 1.83 μg and 1.62 μg for the sedentary.

Table III indicates that no difference existed between the means of the three groups in resting total catecholamine excretion.

TABLE III: ANALYSIS OF VARIANCE ON THE RESTING EXCRETION OF CATECHOLAMINES

Source	S.S.	D.F.	M.S.	F.
Between	115	2	57.1	.51*
Within	15,369	14	110.0	
Total	15,511	16		

* Not significant at .05 level

Tables IV and V indicate the analysis of covariance for the three groups and the effect of exercise on the catecholamine excretion. Resting excretion was used as a criterion. Significant differences were obtained between the means of the total catecholamine excretion from rest ($p < .01$) to maximal and from maximal to submaximal ($p < .01$) work loads. From rest to submaximal the difference in means was significant ($p < .01$). The difference in means within the groups was also significant ($p < .05$).

TABLE IV: ANALYSIS OF COVARIANCE OF CATECHOLAMINE EXCRETION
AFTER MAXIMAL AND SUBMAXIMAL EXERCISE FOR THE THREE
GROUPS PER TOTAL VOLUME EXCRETION

Source	S.S.	D.F.	M.S.	F.
Groups	-271,318	2	-135,659.30	8.01*
Exercises	-412,121.57	1	-412,121.57	24.80*
Interaction	-194,856.06	2	-87,426.03	5.35**
Error	-448,836.70	27	-16,623.53	

* Significant at .01 level

** Significant at .05 level

TABLE V: ANALYSIS OF COVARIANCE OF CATECHOLAMINE EXCRETION
AFTER MAXIMAL AND SUBMAXIMAL EXERCISE FOR THE THREE
GROUPS PER 10 ml. URINE EXCRETION

Source	S.S.	D.F.	M.S.	F.
Groups	-2,607,878.22	2	-1,303,939.11	14.81*
Exercises	-2,362,701.47	1	-2,362,701.47	26.84*
Interaction	-2,344,008.95	2	-1,172,004.47	13.31*
Error	-2,376,269	27	88,009.99	

* Significant at .01 level

It is evident that significant differences exist between the means when urinary catecholamines are used as total excreted or as 10 ml. excretion.

TABLE VI: CATECHOLAMINE EXCRETION FOR ACTIVE, SEMI-ACTIVE
AND SEDENTARY GROUPS AFTER MAXIMAL EXERCISE*

Subject	Active	Semi-Active	Sedentary
1	0.04	0.40	0.10
2	0.13	0.19	0.19
3	0.13	0.14	0.16
4	0.54	0.20	0.46
5	0.52	0.12	0.13
6	0.21	-	0.03
$\bar{x}$	0.26	0.21	0.18

* All values are given in $\mu\text{g}/10 \text{ ml.}$

From tables IV, V and VI a marked difference existed between the means of the three groups for excretion after the exercise ($p < .01$). The excretion ranged from .04 - .54 $\mu\text{g}/10$ ml. for the catecholamines. The volumes of urine excreted after the maximal exercise ranged from 32 - 90 ml. and the means were: active 62 ml., semi-active 50 ml. and sedentary 62 ml. When the urinary excretion of catecholamines/10 ml. was compared to the maximal exercise excretion/10 ml. it was significantly less ($p < .01$). However, when the total catecholamine excretion was considered no differences were found for the active group, indicating an increase of catecholamine excretion per ml. of urine. The other groups showed a decreased catecholamine excretion ($p < .01$).

TABLE VII: CATECHOLAMINE EXCRETION FOR ACTIVE, SEMI-ACTIVE AND SEDENTARY GROUPS AFTER SUBMAXIMAL EXERCISE*

Subject	Active	Semi-Active	Sedentary
1	0.28	0.42	0.11
2	0.56	0.34	0.34
3	0.37	0.96	0.46
4	0.98	0.48	1.14
5	1.26	0.38	0.45
6	0.40	-	0.13
$\bar{x}$	0.64	0.52	0.44

* All values are given in $\mu\text{g}/10$ ml. urine

Tables IV, V and VII indicate that the submaximal exercise produced much higher excretions when compared to the resting and maximal exercise levels ($p < .01$). The range for the excretion after submaximal exercise was from

.11 - 1.26 $\mu\text{g}/10\text{ ml.}$ The urinary excretion for the submaximal exercise ranged from 36 - 127 ml. with means for the groups, active 79 ml., semi-active 43 ml. and sedentary 77 ml. urine.

Total catecholamine excretions after submaximal exercise for the three groups were as follows: active 5.26 μg , semi-active 2.71 μg and sedentary 3.61 μg . Significant increases were found for all three groups, as well as significant differences between the active group and the other two groups.

Table VIII gives the mean urinary catecholamine excretion for the subjects as per 10 ml. urine and total amount excreted during the exercises.

TABLE VIII: MEAN/10 ml. AND MEAN TOTAL URINARY CATECHOLAMINE EXCRETION FOR ACTIVE, SEMI-ACTIVE AND SEDENTARY GROUPS AFTER REST, MAXIMAL AND SUBMAXIMAL EXERCISE.

Group	Rest		Maximal		Submaximal	
	$\mu\text{g}/10\text{ ml.}$	T./ $\mu\text{g.}$	$\mu\text{g}/10\text{ ml.}$	T./ $\mu\text{g.}^*$	$\mu\text{g}/10\text{ ml.}$	T./ $\mu\text{g.}^{**}$
Active	0.34	1.54	0.26	1.61	0.64	5.26
Semi-Active	0.28	1.83	0.21	1.30*	0.51	2.71
Sedentary	0.18	1.62	0.17	1.21	0.44	3.61

* Significant decrease from resting

** Significant increase from resting ($p < .01$)

CHAPTER V

DISCUSSION OF RESULTS

Catecholamines, when administered intravenously, have been shown to cause certain changes in the body, such as: increased heart rate, increased cardiac output, dilation and constriction of blood vessels, the mobilization of free fatty acids, increased blood glucose and an increase in the oxygen uptake (9, 12, 16, 25). During exercise these changes help the body to adapt to the demands of the exercise. The greater the demands, the more catecholamines must be secreted to satisfy the need of the body. The demand for these changes varies in individuals depending on their level of training and on the time they have exercised.

In the present study the results indicate that although resting catecholamine excretion levels are similar in active and non-active groups, after submaximal exercise an increase occurs, but a decrease occurs after maximal only for the non-trained groups. The increased excretion in the urinary catecholamines was derived from the increased output to the circulatory system (72, 73).

There are two possibilities for the source of the increased catecholamine excretion, namely SNS activity and the adrenal medulla. Although there was a decrease of the urinary catecholamine excretion during maximal exercise, increases in the blood catecholamines during this type of

exercise have been found (30, 67). The SNS is responsible for the activation of smooth muscle in the blood vessels, bronchioles, skin and spleen (58). During exercise there is an increased SNS activity (73) as well as an increased adrenal medulla activity (73). While the SNS is being activated an increase in NE release during exercise is inevitable. The adrenal medulla would release an increased amount of E into the bloodstream.

Exercise and the ability to perform for a prolonged time is dependant on the availability of the energy sources and the level of physical fitness. The latter may be related to the use of fat as a source of energy (28). Norepinephrine is a prime adipokinetic agent for the mobilization and release of free fatty acids (28, 64). During the aerobic stage of oxygen utilization lactic acid is oxidized to pyruvic acid. This occurs during submaximal exercise (60) and exhaustion will depend on the availability of oxygen. Catecholamines have been reported to increase the oxygen uptake (60).

After maximal exercise, a significant difference was found between the active group and the other two groups for urinary catecholamine excretion ($p < .01$). The active group excreted the most catecholamine, possibly because of an adaptation of the body to exercise and training. This increased catecholamine excretion of the active group was indicative of increased catecholamine release from the adrenal medulla and the SNS as an adaptation mechanism for

the increased activity. The other groups were apparently not able to increase the catecholamine excretion to meet the increased needs of the body. This could be due to the shut-down of the kidney (8, 31, 81) either by decreasing the blood flow through the kidney or by decreasing the filtration rate due to constriction. Even though the decrease was marked in the urinary excretion of the catecholamines, an increase in blood catecholamines during maximal exercise has been previously noted (30, 67). The active group did not demonstrate the decreased urinary catecholamine excretion after maximal exercise suggesting an increased catecholamine secretion. As has been previously mentioned, the greatest increase would be the NE (73, 74).

After submaximal exercise a significant difference existed between the excretion level of the three groups ($p < .01$). Here again, the active group excreted more catecholamines than the semi-active group or the sedentary group. During this type of exercise the catecholamine excretion level depended on the level of training of the individual. When a highly trained athlete, as in the case of the active group, performs a submaximal exercise free fatty acids and glucose are utilized for energy more readily (28, 64). The oxygen was partially used to metabolize the lactic acid to pyruvic acid and to oxidize fat (53). Catecholamines are also mobilizers of free fatty acids (28, 64). These processes are more evident in active persons than in inactive persons (28) thus causing an increase in the cate-

cholamine excretion in the urine.

After submaximal exercise kidney function does not shut down to the extent found during maximal exercise (59). The free fatty acids are the prime source of energy for muscular exercise (28, 64). For the increased release in the free fatty acids from the adipose tissue an increase must first occur in the adipokinetic agents. The prime mobilizing mechanism for free fatty acids has been found to be NE (12, 64). The three groups demonstrated significant increases in the urinary catecholamine excretion levels ($p < .01$). However, the active group had a significantly greater excretion than the other two groups. There appears to be a correlation between urinary catecholamine excretion and work performance since the active group was able to work at a greater load for a longer period of time than the non-trained groups. Relationships between urinary catecholamine excretion and MVO_2 have previously been reported (73).

During the assaying process, there were many chemical steps involved where the catecholamines could be lost. While adjusting the pH to 4 some of the catecholamines could be lost. Although this step was introduced to remove interfering substances (51) catecholamines could be adsorbed to the alumina. In other methods (58, 82) the conversion of the catecholamines to adrenochrome and noradrenochrome was accomplished with an acid, in the presence of iodine (51). Because there is a trace of iodine present in the urine (18) and hydrochloric acid was added, this could be a cause

of the loss of catecholamines.

By using a magnetic stirrer (79) the recovery was much lower than when using a glass rod with a rubber tip to stir the urine during the adjustment process.

The pH of the urine was adjusted to 8 with sodium hydroxide. Because the catecholamines are very unstable at a pH of 8 a loss during this stage was inevitable (83). This stage must be carried out very quickly to prevent excessive loss. For the best adsorption the pH must be kept between 8 and 8.6. The addition of more than a small amount of distilled water, as in rinsing the apparatus was found to increase the loss.

Several temperature programs were tried. The best separation of the urine extracts, and therefore the most appropriate temperature for this study, was from 150 - 220 °C. at a rate of 5°C per minute.

Even though the gas chromatographic determination of catecholamines in the urine is fairly new, it is felt that this can be developed into a reliable method for the assay. The fluorometric method is not completely specific. The urine extracts, as shown by the chromatograms, contain a complex mixture of substances. It is possible that some of these may interfere with the fluorometric assay. The other

peaks on the chromatograms were not identified by the researcher, but would include the catecholamine metabolites. Further study on this topic is under way.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The basic purpose of this study was to compare the urinary excretion of catecholamines during rest, maximal and submaximal exercise in active, semi-active and sedentary subjects. A secondary purpose was to establish and use a new, and more reliable method of assaying catecholamines in the urine.

Seventeen subjects from the University of Alberta were divided into three groups. Three tests were administered on a Monarch Bicycle ergometer; the first to determine an approximate MVO_2 , the second, a maximal test at 100% MVO_2 and the third, a submaximal test at 70% MVO_2 . Resting urine samples were obtained prior to the first test and ten minutes after the completion of each exercise. The subjects were asked to void an hour before obtaining the resting samples and an hour prior to the exercise.

Significant decreases were found for urinary catecholamine excretion from resting to maximal exercise for the non-active group. No difference was evident for the active group. After submaximal exercise significant increases were found for all groups: however, the active group excreted significantly greater amounts of catecholamines than the non-active groups.

The recovery obtained by using the new gas chromato-

graphic method was 64%. The catecholamines were adsorbed onto alumina at a pH of 8 - 8.6, then eluted from the alumina with acetic acid. The free catecholamines were dried and converted to trimethylsilyl derivatives and injected into the column of the gas chromatograph.

Conclusions. (1) It can be concluded that urinary catecholamine excretion is greater in active than in semi-active or sedentary subjects after maximal and submaximal work load. (2) It is possible, using a gas chromatograph, to obtain a reliable estimate of the urinary catecholamine excretion, in conjunction with a suitable extraction procedure.

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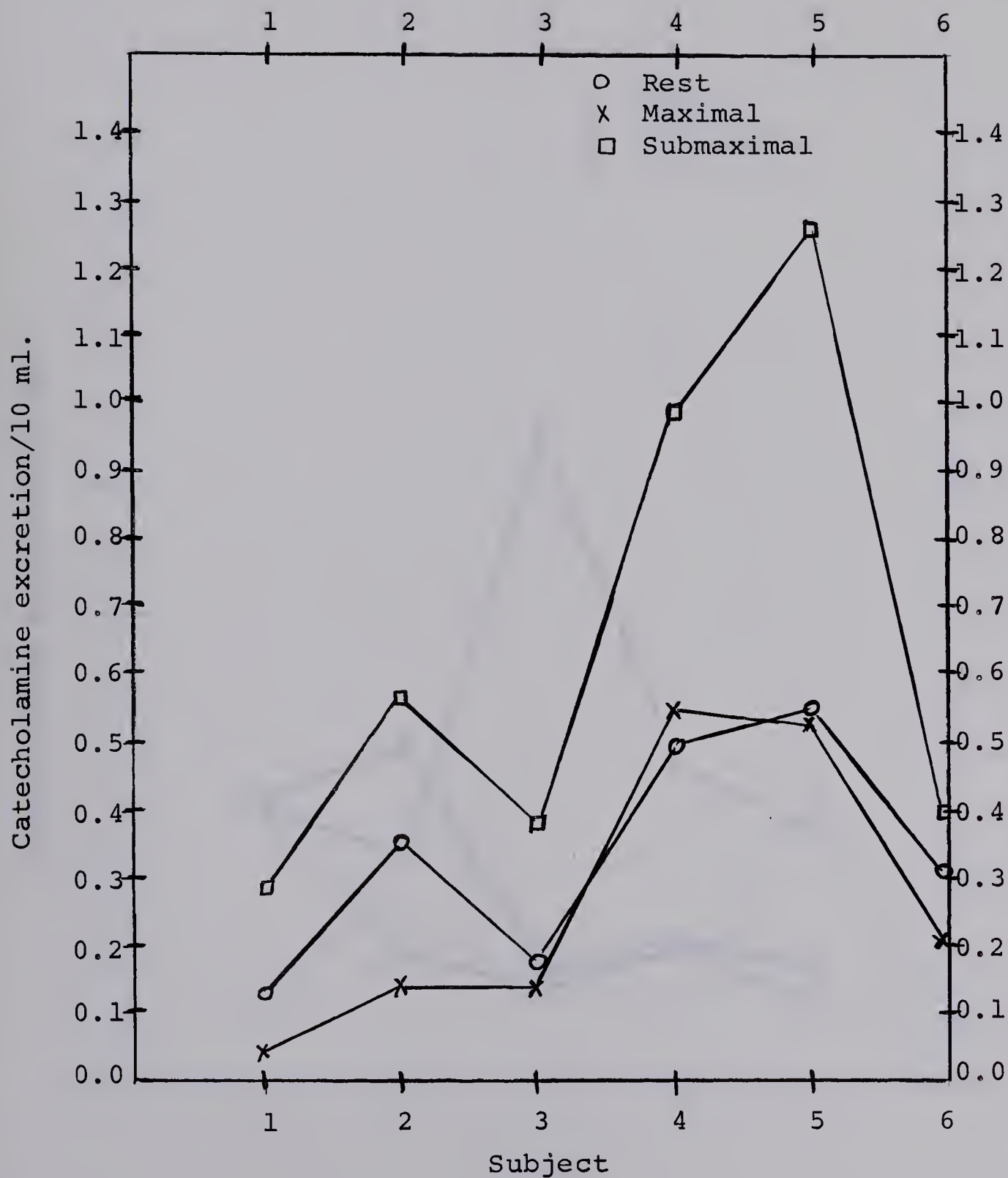
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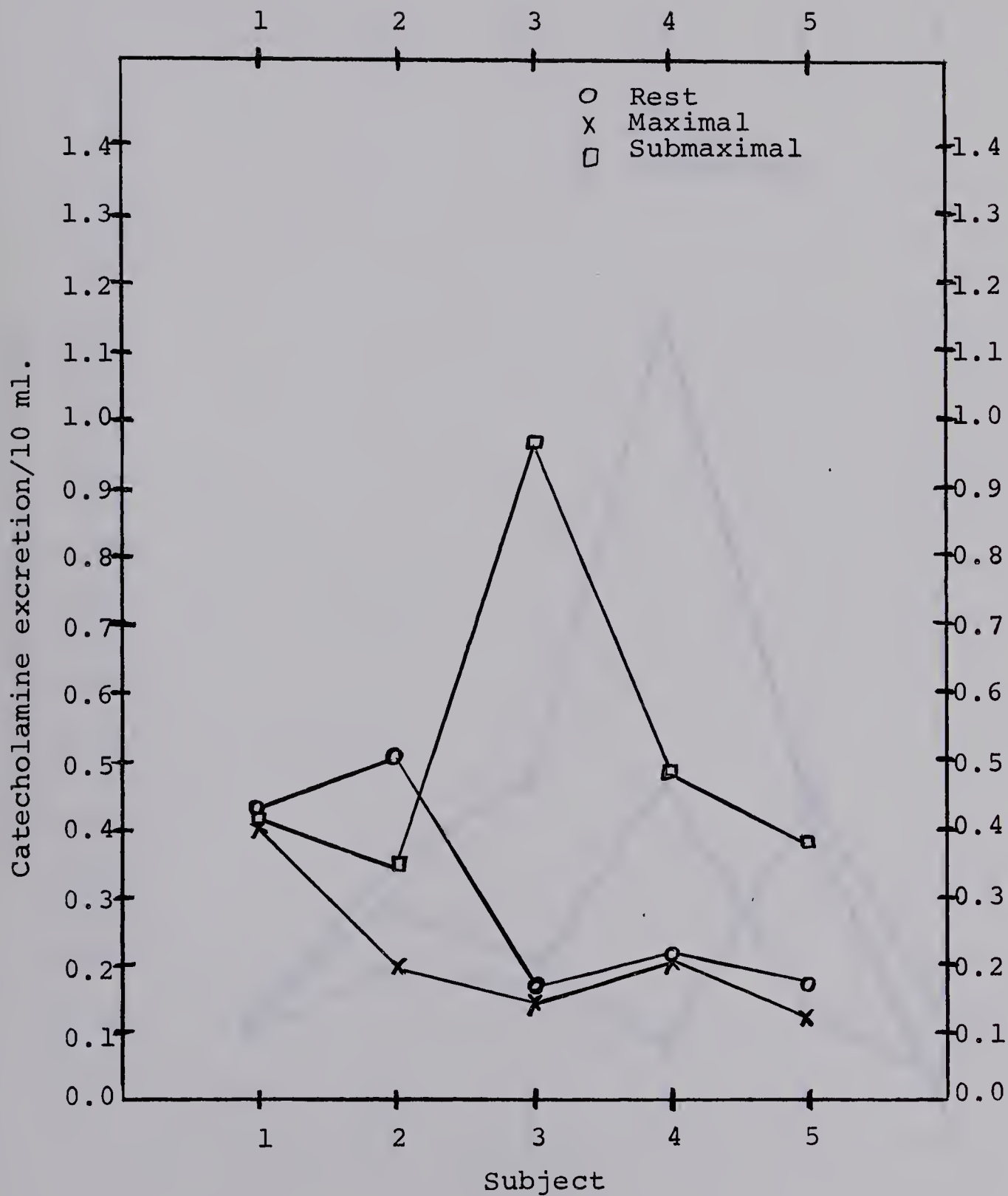
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APPENDIX

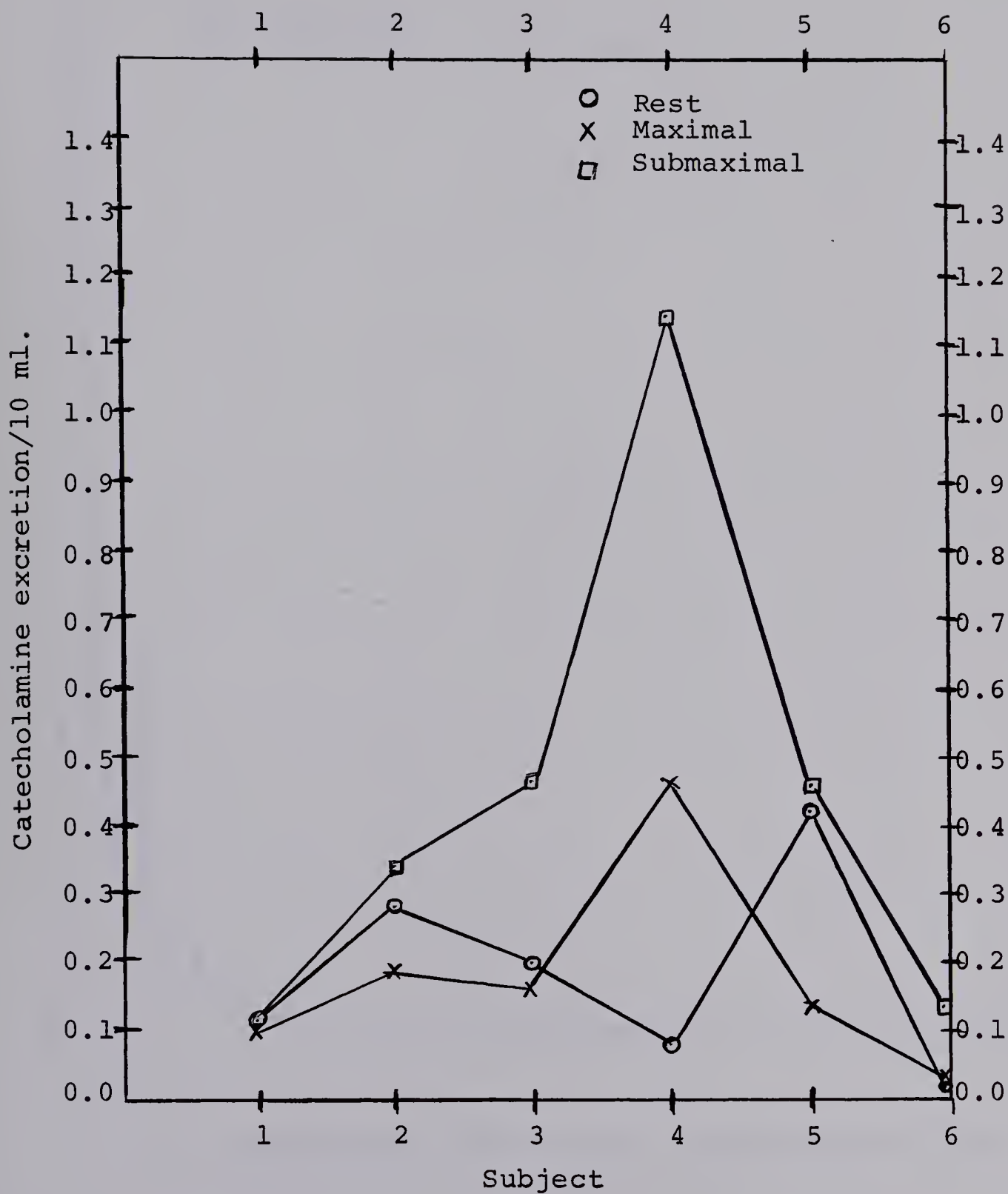




1. CATECHOLAMINE EXCRETION FOR ACTIVE GROUP
DURING REST, MAXIMAL AND SUBMAXIMAL EXERCISE



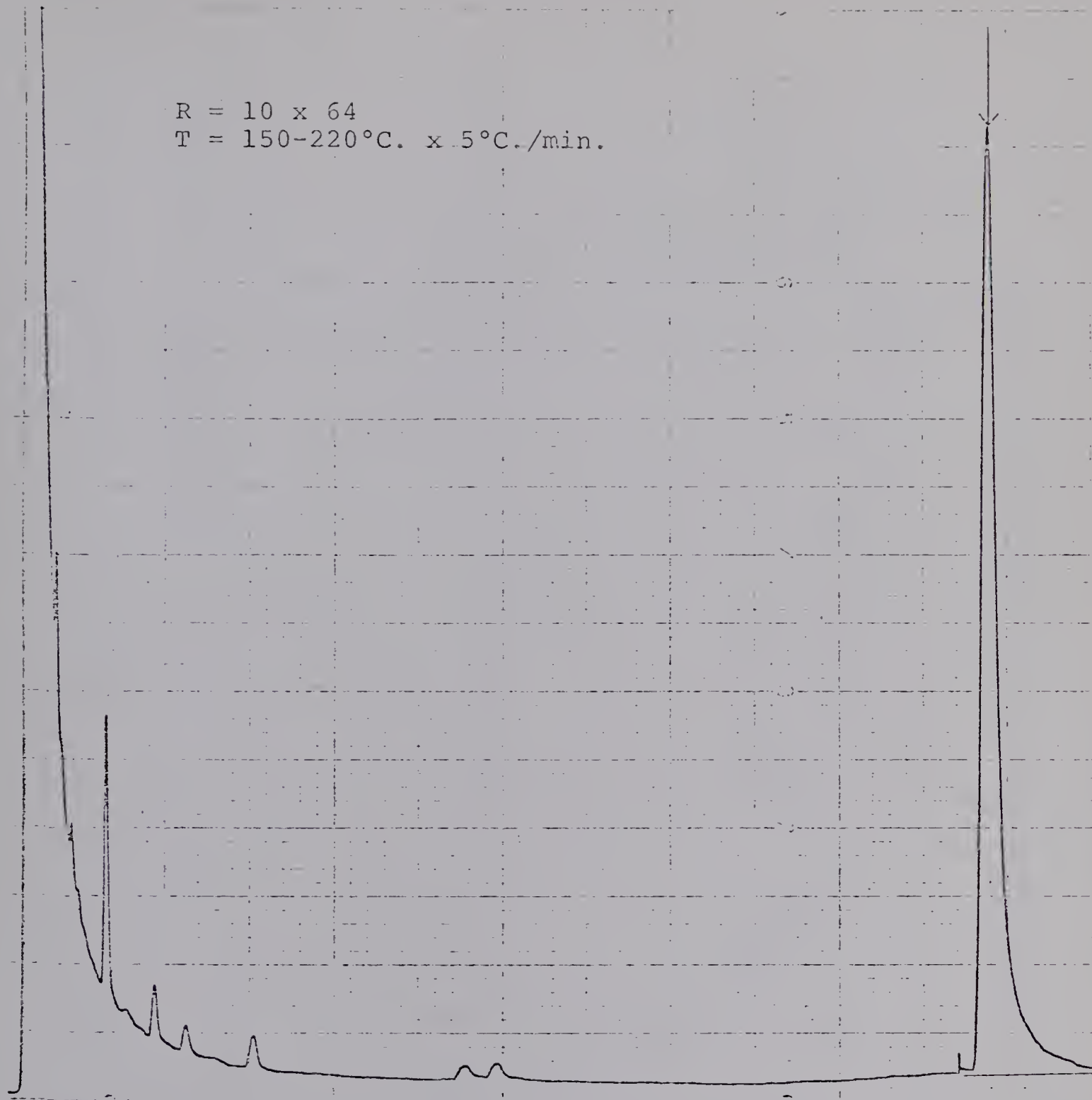
2. CATECHOLAMINE EXCRETION FOR SEMI-ACTIVE GROUP DURING REST, MAXIMAL AND SUBMAXIMAL EXERCISE



3. CATECHOLAMINE EXCRETION FOR SEDENTARY GROUP
DURING REST, MAXIMAL AND SUBMAXIMAL EXERCISE

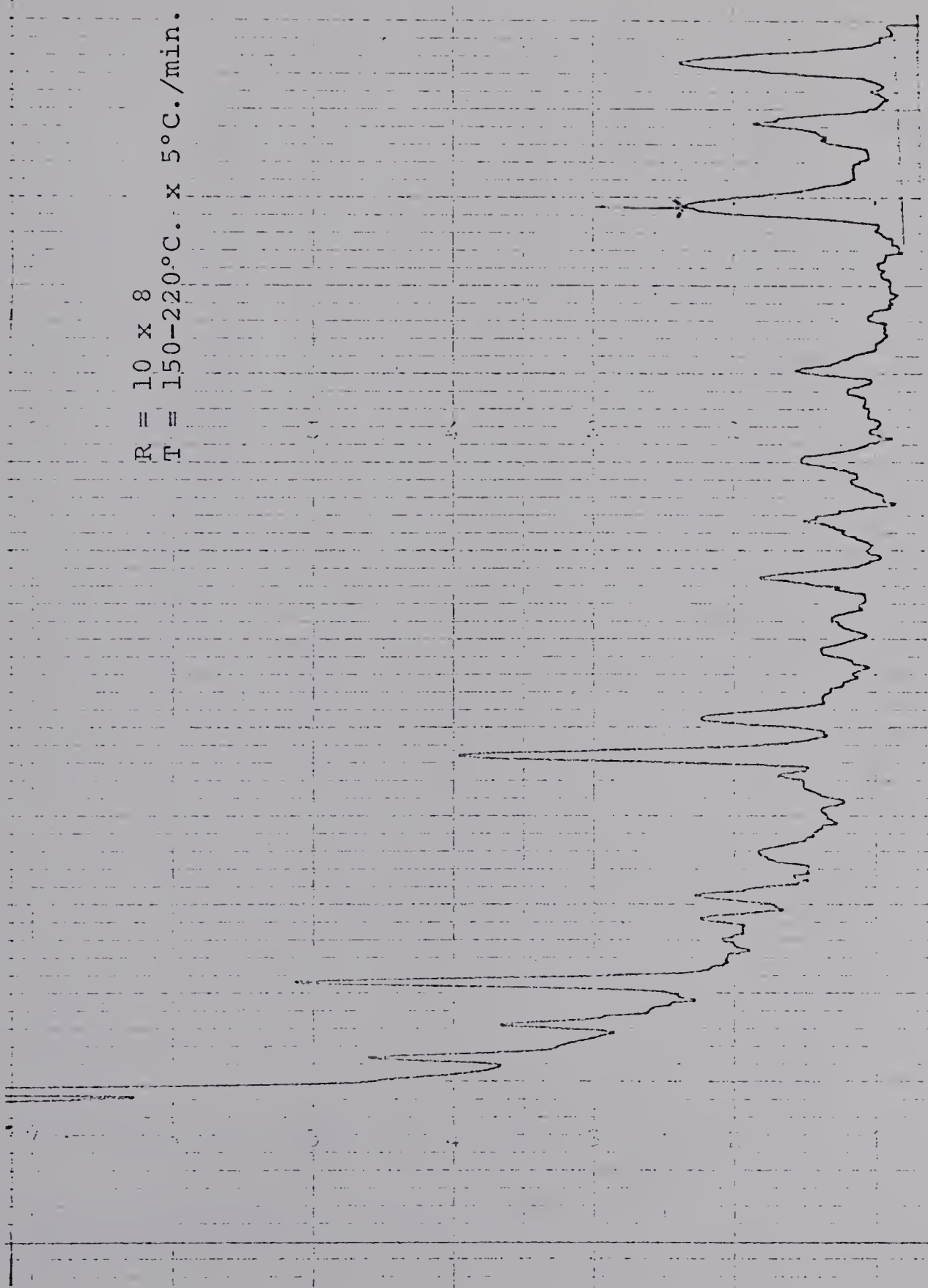
R = 10 x 64

T = 150-220°C. x 5°C./min.

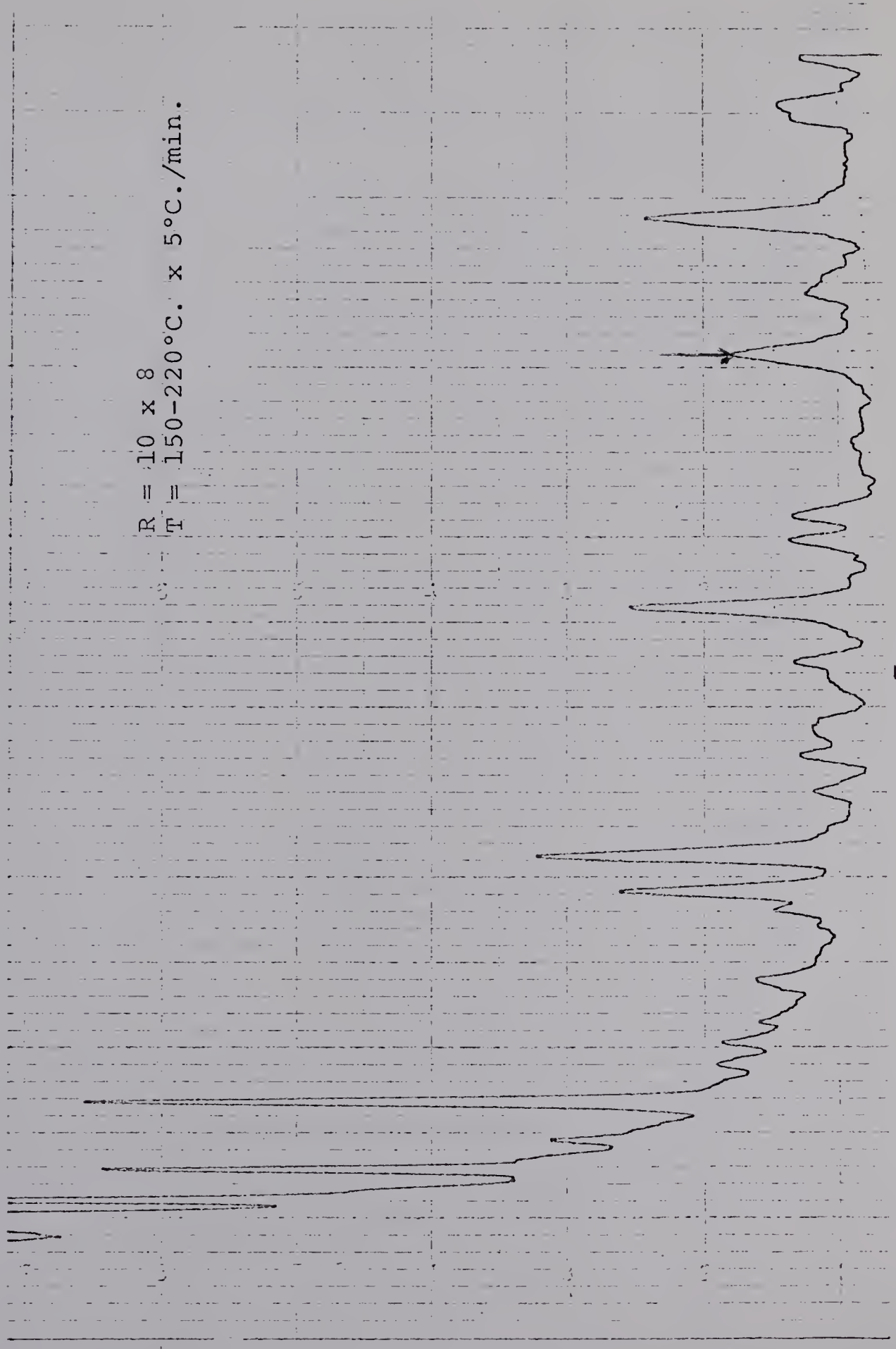


4. CHROMATOGRAM FOR STANDARD CATECHOLAMINE VALUE

R = 10 x 8
T = 150-220°C. x 5°C./min.



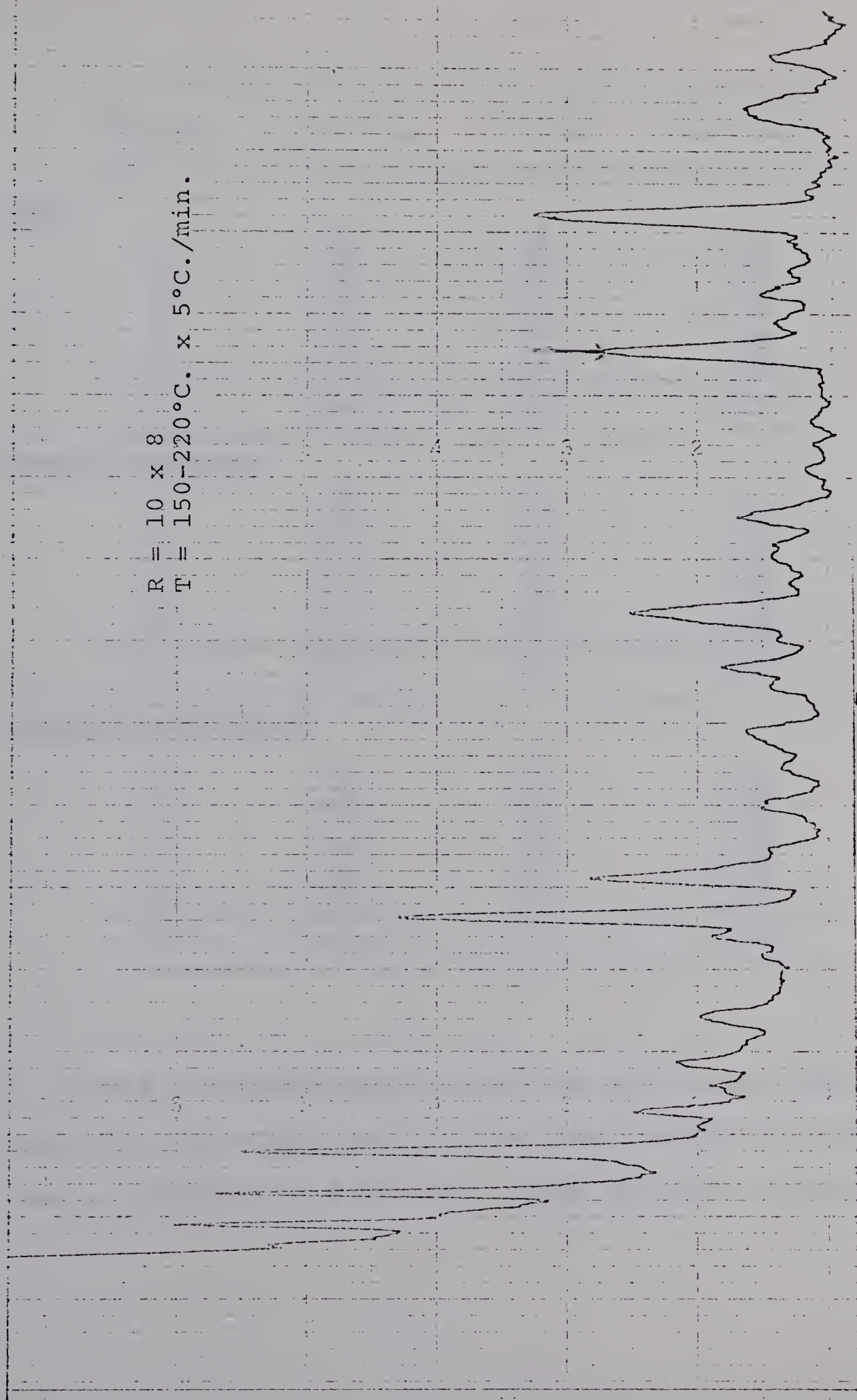
5. CHROMATOGRAM FOR RESTING CATECHOLAMINE VALUE



R = 10 x 8
T = 150-220°C. x 5°C./min.

6. CHROMATOGRAM FOR MAXIMAL EXERCISE CATECHOLAMINE VALUE

R = 10 x 8
T = 150-220°C. x 5°C./min.



7. CHROMATOGRAM FOR SUBMAXIMAL EXERCISE CATECHOLAMINE VALUE

Subject	$\mu\text{g}/10 \text{ ml.}$	$\text{vol}/\text{ml.}$	$\text{vol.} \times \mu\text{g.}$
REST			
1	.13	53	.69
2	.35	58	2.03
3	.17	28	.48
4	.50	74	3.70
5	.55	25	1.38
6	.31	30	.93
$\bar{x}$	.34	45	1.54
MAXIMAL EXERCISE			
1	.04	34	.14
2	.13	66	.75
3	.13	54	.61
4	.54	46	3.02
5	.52	60	3.12
6	.21	69	1.44
$\bar{x}$	.26	62	1.61
SUBMAXIMAL EXERCISE			
1	.28	127	3.56
2	.56	52	2.91
3	.37	69	2.55
4	.98	92	9.02
5	1.26	95	11.97
6	.40	38	1.52
$\bar{x}$	.64	79	5.26

1. TABLE OF CATECHOLAMINE EXCRETION AND VOLUME URINE EXCRETION FOR ACTIVE GROUP DURING REST, MAXIMAL AND SUB-MAXIMAL EXERCISE AS PER 10 ml. URINE AND TOTAL EXCRETION

Subject	$\mu\text{g}/10 \text{ ml.}$	$\text{vol}/\text{ml.}$	$\text{vol.} \times \mu\text{g.}$
REST			
1	.43	87	3.74
2	.50	42	2.10
3	.16	45	.72
4	.21	44	.92
5	.17	98	1.66
$\bar{x}$	.28	64	1.83
MAXIMAL EXERCISE			
1	.40	73	2.92
2	.19	65	1.24
3	.14	70	1.00
4	.20	35	.70
5	.12	56	.67
$\bar{x}$	.21	59	1.36
SUBMAXIMAL EXERCISE			
1	.42	60	2.52
2	.34	87	2.96
3	.96	33	3.17
4	.48	59	2.83
5	.38	54	2.05
$\bar{x}$	.51	43	2.71

2. TABLE OF CATECHOLAMINE EXCRETION AND VOLUME URINE EXCRETION FOR SEMI-ACTIVE GROUP DURING REST, MAXIMAL AND SUBMAXIMAL EXERCISE AS PER 10 ml. URINE AND TOTAL EXCRETION

Subject	$\mu\text{g}/10 \text{ ml.}$	$\text{vol}/\text{ml.}$	$\text{vol.} \times \mu\text{g.}$
REST			
1	.11	83	.91
2	.29	93	2.70
3	.20	109	2.18
4	.08	88	.70
5	.42	75	3.15
6	.02	38	.08
$\bar{x}$	.18	81	1.62
MAXIMAL EXERCISE			
1	.10	90	.90
2	.19	88	1.67
3	.16	32	.51
4	.46	73	3.35
5	.13	57	.74
6	.03	32	.10
$\bar{x}$	.17	62	1.21
SUBMAXIMAL EXERCISE			
1	.11	99	1.09
2	.34	83	2.82
3	.46	36	1.66
4	1.14	95	10.83
5	.45	100	4.50
6	.13	49	.64
$\bar{x}$	.44	77	3.61

3. TABLE OF CATECHOLAMINE EXCRETION AND VOLUME URINE
EXCRETION FOR SEDENTARY GROUP DURING REST, MAXIMAL AND
SUBMAXIMAL EXERCISE AS PER 10 ml. URINE AND TOTAL EXCRETION

Subj.	Age	Weight	Height	70% MVO ₂	Initial Load	Max.Time (sec.)	Submax. Load	Submax. Time
ACTIVE								
1	21	160	70	2.1	±900	180	±600	70
2	21	178	71½	3.0	±1200	170	±700	55
3	21	133	70	3.5	±900	138	±800	60
4	19	154	71	1.7	±1200	163	±600	140
5	20	154	74	1.9	±900	140	±600	80
6	22	159	66½	2.1	±1200	141	±600	52
$\bar{x}$	21	157	70	2.3		155	650	76
SEMI-ACTIVE								
1	23	150	70	2.1	±900	160	±600	19
2	18	155	73	2.1	±900	162	±600	49
3	18	148	68	1.6	±1200	134	±600	76
4	18	170	67	1.6	±900	143	±600	15
5	17	162	70	3.2	±1200	144	±700	50
$\bar{x}$	19	157	69.5	2.1		158	620	34
SEDENTARY								
1	23	154	69	1.8	±700	140	±600	60
2	23	150	69	1.6	±900	165	±600	40
3	23	160	74	1.6	±800	154	±600	35
4	24	170	70	1.7	±800	143	±600	55
5	22	145	70	1.4	±600	165	±600	100
6	20	170	73	1.9	±900	191	±600	75
$\bar{x}$	23	158	71	1.6		159	600	61

4. TABLE OF AGE, WEIGHT, HEIGHT, MVO₂, INITIAL MAXIMAL LOAD, MAXIMAL TIME, SUBMAXIMAL LOAD AND SUBMAXIMAL TIME.

PHENYLALANINE ————— Tyrosine ————— TYROSINE ————— The tyrosine will go from the blood to the S.N. fibres by active transport, or to the cells in the adrenal medulla and other organs in the body

|

Tyrosine Hydroxylase

↓

DIHYDROXYPHENYLETHYLALANINE ← ————— To the mitochondria where the Dopa is decarboxylated by the enzyme Decarboxylase ————— DIHYDROXYPHENYLALANINE
(Dopamine) (Dopa)

In the cytoplasm the dopamine is converted by an enzyme Beta-oxidase. In this form it binds with the granulated vesicle and is stored in the chromaffin cells

|

Beta-Oxydase

↓

NOREPINEPHRINE ————— N.E. can now be released from the granulated vesicle into the bloodstream or methylated by an enzyme to E. in the cytoplasm and released as such. (Phenylethanolamine-N-Methyltransferase) ————— EPINEPHRINE
Can be excreted in the Urine Can be excreted in the Urine

In the liver and kidneys, which are rich with an enzyme Catechol-O-Methyltransferase (C.O.M.T.), the E. and N.E. can be converted to:

NORMETANEPHRINE
(O-Methyl N.E.)



Or it can be changed in the blood by Monoamine Oxidase to:

VANILYL MANDELIC ACID
in the Urine

or

METHOXY HYDROXY PHENYL GLUCOL
in the Urine

Normetanephine can now be metabolized to conjugated derivatives by enzyme Monoamine Oxidase



NORMETANEPHRINE CONJUGATE
Can be excreted in the Urine

METANEPHRINE
(O-Methyl E.)



Metanephine can now be metabolized to conjugated derivatives by enzyme Monoamine Oxidase

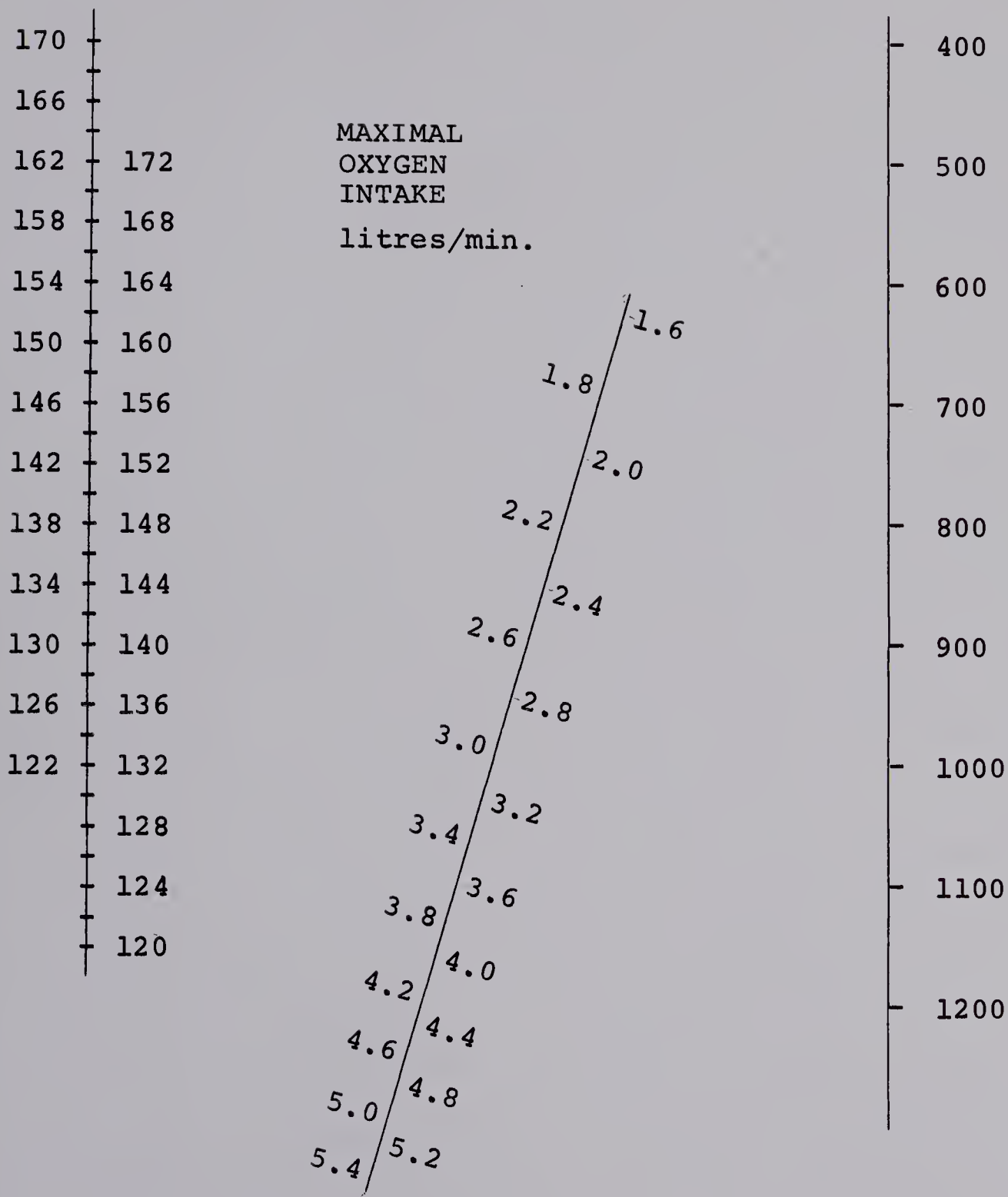


METANEPHRINE CONJUGATE
Can be excreted in the Urine

PULSE RATE

WORK LEVEL

kgm/min.



ASTRAND-RHYMING NOMOGRAM (6)

B29899